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Female choice for sick males over healthy males: Consequences for offspring

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Abstract

Sexual selection theory indicates that ornament expression in males is in close relation to their condition. This "honesty" relationship serves as the basis for female choice: Females would mate with healthy males over sick males after assessing male ornament signal expression and derive benefits for their progeny. Here, we investigated female mate choice for infected and non-infected males, male survival after infection (to corroborate the negative effect of infection), and fitness consequences of female preferences using *Tenebrio molitor* beetles. Male infection was produced having two types of challenges as follows: males infected with entomopathogenic fungi and males infected with nylon implants. Similar to previous studies, we corroborated that females preferred fungus-infected males over positive control, negative control, and nylon-challenged males. Survival was the lowest for fungus-treated males followed by nylon-treated and control males. Females mated with fungus-treated males laid fewer and smaller eggs, and the laid eggs had less lipid content with a reduced eclosion success compared to females mated with non-challenged males. Our interpretation is that fungus-treated males invested their energetic resources to increase their attractiveness at the risk of survival, in a terminal investment fashion. Females, however, would have corrected their choice by investing less in their offspring.

KEYWORDS
female choice, male signal, offspring viability, *Tenebrio molitor*

1 | INTRODUCTION

The handicap principle (Zahavi, 1975) proposes that male sexual ornaments are honest signals that females use to assess quality of potential mates (Andersson & Simmons, 2006; Bradbury & Vehrencamp, 2011; Palau-Daval, Gardette, & Joly, 2018). One way this "honesty" relationship can be interpreted is that signals reliably indicate the ability of organisms to resist parasites (Balenger & Zuk, 2014; Hamilton & Zuk, 1982). According to this idea, honesty via male ability to deal with parasites relies on two premises. On one hand, parasites affect hosts' survival if hosts are not capable to resist them (e.g., Ebert, Lipsitch, & Mangin, 2000; Davies, Fairbrother, & Webster, 2002). On the other hand, the maintenance and expression of the immune machinery to deal with parasites are energetically costly (e.g., Fellowes & Godfray, 2000; Sol, Jovani, & Torres, 2003; Ardia, Gantz, Schneider, & Strebel, 2012). In this fashion, signals are considered honest indicators of animal condition (Bradbury & Vehrencamp, 2011) and this is the reason why females use their expression when choosing a mate (Andersson & Simmons, 2006; Hamilton & Zuk, 1982). Such choice will grant females to secure parasite-resistant genes that can be passed on to their offspring.

According to life-history theory, organisms adjust their investment in current reproduction considering the gains and future events of reproduction (Ratikainen & Kokko, 2009; Stearns, 1992). An important factor that affects this investment is the quality of mates. For the case of females, it is expected that these can adjust their reproductive investment depending on male attractiveness (Burley, 1986; Harris & Uller, 2009; Sheldon, 2000). Such adjustment can be seen as maternal effects that can alter the strength of sexual selection and male fitness (reviewed by Harris & Uller, 2009). In particular, the differential allocation hypothesis indicates a positive relation between male quality and maternal investment: That is, a reduced resource allocation by females when mated with males in poor condition (Sheldon, 2000). Ways by which females can adjust their resources can be expressed in different ways, many of these directly related to egg traits such as an increase in egg size (e.g., Kolm, 2001), number (e.g., Locatello & Neat, 2005), antibody concentration (e.g., Saino, Romano, Ferrari, Martinelli, & Moller, 2003), and lipid and protein content (e.g., Braga et al., 2010).

One study system where female choice based on male ability to resist pathogens and female resource allocation to offspring can be investigated is that of *Tenebrio molitor* beetles. In this species, males signal their quality to females by producing volatile pheromones (mainly composed of 3-dodecenyl acetate, Bryning, Chambers, & Wakefield, 2005) and a set of sex-specific cuticular hydrocarbons (Nielsen & Holman, 2012). However, studies where males have been immune-challenged to see female choice patterns for male pheromones have found opposite findings: While some studies indicated that males with low levels of infection or no infection were more attractive (Worden & Parker, 2005; Worden, Parker, & Pappas, 2000), other studies determined that immune-challenged males were more attractive (Kivleniece, Krams, Daukšte, Krama, & Rantala, 2010; Krams et al., 2011; Nielsen & Holman, 2012; Sadd et al., 2006). Following these controversial results, in this paper we have set the question of whether females choose healthy males over sick males. Note, however, that our study differs from previous examinations of female choice in the same study system for several reasons. Apart from nylon challenges, we also used real pathogens in the same experimental design. The reason for this is that compared with an artificial challenge, a real pathogen can multiply within its host thereby intensifying its debilitating effects (Moreno-García, Córdoba-Aguilar, Condé, & Lanz-Mendoza, 2013). As a matter of fact, a study in this animal showed that a fungal challenge induced a more rapid mortality than a nylon implant (Krams, Daukste, Kivleniece, Krama, & Rantala, 2013). For these reasons, we used *Metarhizium robertsii*—an entomopathogenic fungus—and nylon implants to infect males. *M. robertsii* (formerly known as *M. anisopliae* var. Anisopliae; Bischoff, Rehner, & Humber, 2009) is widely distributed in soil and has been used as a biological control agent (Faria & Wraight, 2007; Maniania et al., 2003; Wang & Feng, 2014). Second, we monitored survival after a challenge, via an experimental design that includes all animals that died in all treatments. This unlike previous studies where mortality was assessed one month following manipulation where a fraction of surviving animals was left aside (Kivleniece et al., 2010; Krams et al., 2011). By removing a fraction of animals, survival estimates may be obscured. Third, we assessed offspring condition and viability of chosen males to examine whether the differential allocation hypothesis applies. For this, we measured (i) the volume and concentration of proteins and lipids of the eggs. Insect egg volume is in general, a good predictor of egg survival (i.e., Sota & Mogi, 1992), development and future size in both the immature and mature stage (i.e., Fox, 1994). On the other hand, while proteins participate in egg immune protection (Gillepie, Kanost, & Trenczek, 1997), lipids can provide energy for growth during extended periods of non-feeding (Arrese & Soulages, 2010); and (ii) number of eggs laid over one week as well as emerging larvae. Our predictions were that (i) females should prefer healthy males over non-healthy males with fungus-treated males being less selected than nylon-treated males; (ii) negative effects of challenge imply that fungus-treated males will be the first to die followed by nylon-treated and control males; (iii) challenged males will sire fewer eggs, with poorer condition (smaller, with less protein and lipid concentration) and/or eclosion success compared to control males.

2 | MATERIAL AND METHODS

2.1 | Insect breeding

Tenebrio molitor larvae were obtained from four different commercial suppliers in Mexico City and the State of Mexico. Larvae were kept at 25 ± 2°C (mean ±standard deviation) in a 12:12-hr photoperiod cycle and fed with wheat ad libitum and apple slices. Pupae were collected, sexed by examination of the eighth abdominal segment (Bhattacharya, Ameel, & Waldbauer, 1970), and individually separated to ensure that all adults were virgin at the moment of experimentation.

2.2 | Fungus cultivation and inoculum preparation

Metarhizium robertsii (ARSEF 2134) was obtained from the entomopathogenic fungi collection of the Agricultural Research Service of the United States Department of Agriculture. Spores were stored in 10% glycerol at −80°C until their use. For preparation of the inoculum, spores were plated on sabouraud dextrose agar (SDA) and incubated at 28°C in darkness. After 15 days of incubation, conidiophores were harvested by scraping them out gently from the plate with a scalpel and suspended in 0.03% Tween 80 (hereafter referred as Tween). The suspension was stirred for 5 min on a vortex and filtered through a cotton mesh to separate out the conidia from the mycelium. Conidia were counted using a Neubauer chamber. The percent viability of the conidia was greater than 95%, and it was estimated using the SDA plate count technique (Goettel & Inglis, 1997).

2.3 | Determination of the LC_{50}

Five groups of 15 males of *T. molitor* each were inoculated with five different concentrations of *M. robertsii* conidia suspended in 10 ml

of Tween (1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia/ml). Inoculation was carried out by immersing insects into the conidial suspensions for five seconds and air-dried in a 9-cm diameter Petri dish with a Whitman No. 1 filter paper at the bottom. A sixth control group was immersed in Tween without conidia and dried in the same way. Insects were placed individually in a cavity of a 12-well plate with wheat and incubated for 10 days at 25°C and 90% humidity after inoculation. Mortality was recorded every 24 hr for ten days and dead insects were incubated at 25°C in a 5-cm diameter Petri dishes with wet filter papers to promote sporulation and confirm fungal infection. That an animal was dead was corroborated by making sure that it remained motionless even after tactile manipulation. All male ages and weights ranged between 12 to 15 days and 0.9 to 0.12 g, respectively.

2.4 | Male challenge treatments

Four treatment groups of sexually mature males (12 to 15 days old; Gerber, 1976) were established in the following way: (i) negative control, non-manipulated animals; (ii) fungus, animals immersed in Tween with 3×10^5 conidia/mL (the approximate LC_{50}) for five seconds; (iii) positive control, animals submerged in Tween for five seconds; and (iv) nylon, animals with three nylon filaments (2-mm long by 0.5-mm diameter) fully inserted between the second and third abdominal sternites. Nylon filaments were previously disinfected by storing them in 96% ethanol for 24 hr. before insertion. All animals were kept for three days before female choice trials.

2.5 | Female choice trials

Five different experiments of female choice were carried out arranging the treatment groups as follows: (i) negative control versus fungus; (ii) negative control versus implant; (iii) negative control versus positive control; (iv) positive control versus fungus; and (v) implant versus fungus. Each experiment consisted in 30 tests (for a total of 150 trials) where a sexually mature, virgin female (20 days old; Gerber, 1976) was allowed to choose between two males in a two-armed olfactometer (Supporting Information video S1). At the beginning of each test, the female was placed for 2 min in the release port for acclimatization. Then, the gate of the release port was opened and the behavior of the female recorded. Males remained restrained at the end of the arms of the olfactometer during the whole test, and an air pump connected to the arms was used to push males' scents to the female. Males were randomly assigned to each arm of the olfactometer. The test was concluded 3 min after opening the gate. Two behavioral responses were recorded as follows: female choice (decision to take right or left olfactometer arm) and the time it took females to choose one of the arms (from port opening until reaching the end of the arm). The observer did not know the experimental origin of all males. The olfactometer was cleaned with ethanol after every replicate to remove the remnants of the chemical signals (Ríos-Delgado et al., 2008). Since this species usually mates in the dark, tests were carried out in a dark room with a red light which cannot be detected by the animal but allowed us to record its behavior (for a rationale see Briscoe & Chittka, 2001).

2.6 | Male survival after challenge

We had the same male experimental groups (negative control, fungus, positive control, and nylon) as indicated above, using 30 virgin males of 12–15 days of age. After manipulation, each animal was individually separated, and mortality was recorded every 24 hr. until all animals died. Similar to LC_{50} determination described above, we corroborated that an animal was dead if it remained motionless after tactile manipulation.

2.7 | Offspring condition and viability

The same five experimental combinations of female choice described above were carried out again but this time the number of tests was increased to reach 20 pairs for each male–female combination (for a total of 200 trials). After tests were performed, each pair (female and chosen male) was transferred to a plastic container (4.6-cm diameter by 4.6-cm height) with 9 grams of commercial wheat flour and maintained at room temperature for 1 week. Our previous experience indicated that such food provision is needed by females to lay eggs (all authors' unpub. data). After this time, 20 mating pairs per treatment (thus having a total of 100 pairs) were randomly selected and 5 eggs per female were randomly separated to measure their volume, protein, and lipid concentration. The remaining 20 mating pairs from each treatment were used to record the number of eggs females laid for one week and eclosion success of these eggs measured as the number of hatching larvae.

2.8 | Egg volume, protein, and lipid concentrations

Soon after being laid, eggs were photographed under a stereoscopic microscope and their volume was depicted using ImageJ (Schneider, Rasband, & Eliceiri, 2012). Volume was calculated using the formula reported by Berrigan (1991): $V = 1/6\ \pi\ w^2\ L$, where w and L are egg width and length, respectively.

Total protein and lipid concentrations were determined using Foray et al.'s (2012) methodology using the same 5 eggs whose volume was measured. Eggs were ground with 200 µl of PBS pH 7.4 using a micropestle. The resultant suspension was centrifuged at 4°C for 10 min at 10,000 RPM. Protein concentration was determined using the Pierce™ BCA Protein Assay Kit (Rockford, IL, USA; Smith et al., 1985). According to this, 10 µl of the sample supernatant were mixed with 40 µl of PBS and 150 µl of the kit reagents and then incubated in a 96-microwell plate for 30 min at 37°C. Bovine serum albumin was used to perform the standard reference curve, and the absorbance was recorded at 562 nanometers in a microplate reader. Lipid concentration was determined by the Van Handel (1985) method. In brief, 180 µl of the sample supernatant were mixed with 1 ml of chloroform-methanol (1:1) and vortexed for 2 min. 100 µl of this sample were taken and heated at 90°C to evaporate the solvent.

Then, 10 µl of sulfuric acid (98%) was added to the sample and incubated at 90°C for 2 min. After cooling, each sample was placed in a 96-microwell plate with 190 µl of phospho-vanillin (1.2 g/L). Glyceryl trioleate was used to perform the standard reference curve, and the absorbance was recorded at 562 nanometers.

2.9 | Statistical analysis

LC_{50} was determined with a Probit analysis using the mortality data at day 5. LC_{50} was estimated at 3.9×10^5 conidia/ml with the 95% confidence interval between 8.11×10^4 and -1.43×10^6 conidia/ml. For the female choice experiment, a *G* test of goodness-of-fit was performed to determine whether there were differences in female preference of the two males tested in the olfactometer. The time it took females to choose a male from each treatment group in these experiments was compared with a Mann–Whitney *U* test since data did not meet the parametric assumptions even after transformation. For the male survival experiment, we used a generalized linear model (GLM) using the proportion of individuals that survived according to time from challenge and experimental treatment. In this GLM, we fitted a quasibinomial error distribution to correct for data overdispersion. We used Tukey tests to compare among treatments. As for egg traits, we first tested if male treatment affected the volume of eggs, so that we used a nested ANOVA having treatment as a fixed factor and female identity as a random factor nested within treatments. A Tukey–Kramer test was used as a post hoc test to observe significant differences between treatments. A Welch's ANOVA was used to compare protein and lipid concentrations according to male origin female mated with, since data did not meet the assumption of homogeneity of variances. A Games-Howell test was used as a post hoc test to detect significant differences. Normality and homoscedasticity were tested with the Shapiro–Wilk and Levene's tests, respectively. To assess whether egg number and eclosion were related to treatment, we used a GLM where we set a Poisson and a binomial distribution for egg number and eclosion success, respectively, to correct for overdispersion. Tukey tests were used to compare differences among treatments. All analyses were carried out in R (R Core Team, 2017), except the Probit analysis, which was carried out in the Polo Plus™ software (LeOra Software, 2002). R packages used were car (Fox & Weisberg, 2011), DescTools (Signorell, 2017), ggplot2 (Wickham, 2009), multcomp (Hothorn, Bretz, & Westfall, 2008), nlme (Pinheiro, Bates, DebRoy, & Sarkar, 2017), Rmisc (Hope, 2013), and userfriendlyscience (Peters, 2017).

3 | RESULTS

3.1 | Female choice

Preferences for males were not equally distributed in all comparisons (Table 1), and so females preferred (i) fungus males over negative control males ($G = 5.795$, $p = 0.008$) and (ii) fungus males over positive control males ($G = 8.398$, $p = 0.001$). No difference emerged between negative control males versus positive control males (G = 5.15, $p = 0.237$), nylon males versus negative control males (G = 0.057, $p = 0.406$) and fungus males versus nylon males (G = 5.15, $p = 0.237$).

TABLE 1 Number of males chosen by females as mates in *Tenebrio molitor* according to five different male combinations

Treatment	Chosen males	*G* test	*p* value
Negative control versus fungus	25 versus 45	5.795	0.008*
Negative control versus nylon	34 versus 36	0.057	0.4060
Negative control versus positive control	38 versus 32	5.15	0.237
Positive control versus fungus	23 versus 47	8.398	0.001*
Nylon versus fungus	32 versus 38	5.15	0.237

Note. The two numbers in the "Chosen males" column refer to the number of males females chose from each respective treatment. For example, 25 negative control males and 45 fungus-challenged males were chosen from the first treatment.

The time it took females to select a male did not differ among groups in all experiments (*p* values for all four Mann–Whitney *U* test-based experiments>0.05).

3.2 | Male survival effects after challenge

Male survival differed across groups ($\chi^2 = 2.05$, $p < 0.001$; Figure 1). Also, there was an interaction between treatment and time from exposure ($\chi^2 = 2.77$, $p < 0.001$). Comparison between groups showed that fungus males died sooner than the other three groups (against nylon implant, $z = 2.01$, $p = 0.04$; against negative control, $z = 7.16$, $p < 0.001$; and against positive control, $z = 2.70$, $p = 0.007$). Conversely, negative control animals took longer to die than the other three groups (against fungus, $z = 7.25$, $p < 0.001$; against positive control, $z = 5.71$, $p < 0.001$; and against nylon implant, $z = -6.18$, $p < 0.001$). Nylon implant animals died sooner than negative control ($z = -6.18$, $p < 0.001$) but had no difference with positive control animals ($z = -0.631$, $p = 0.921$).

3.3 | Offspring condition and viability

There were differences in egg volume across treatments (nested ANOVA: $F_{3,36} = 8.04$, $p < 0.001$; Figure 2). Tukey–Kramer post hoc tests revealed that the eggs sired from fungus males were significantly smaller than those sired by positive control and implant male groups. However, eggs from fungus males were not different than those of the negative control group (Figure 2).

There were no significant effects of male treatment on total protein concentration (Welch's ANOVA, $F_{3,36} = 1.41$, $p = 0.256$). However, total lipid content was significantly different among treatments (Welch's ANOVA: $F_{3,36} = 12.38$, $p < 0.001$; Figure 3). A Games-Howell test showed that eggs sired by males of the positive control ($z = 5.81$, $p < 0.001$), implant ($z = 4.38$, $p < 0.001$), and fungus treatment ($z = 3.91$, $p < 0.001$) groups had less lipid content

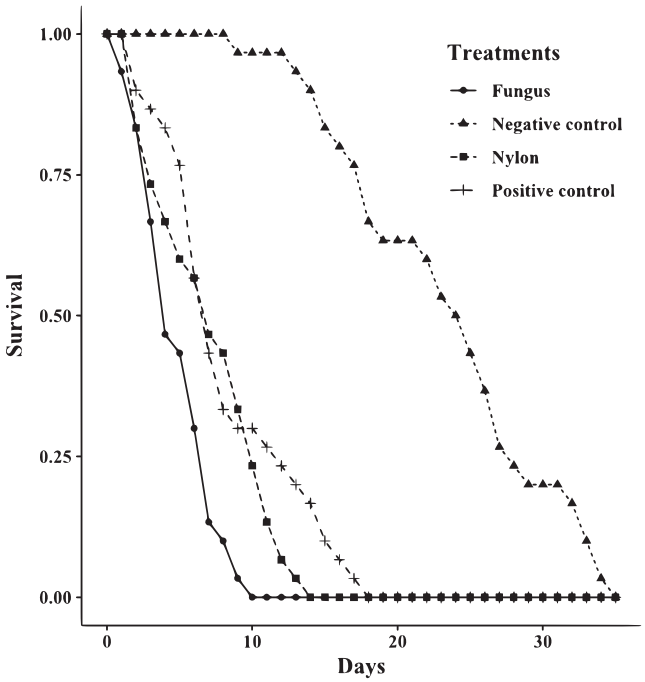

FIGURE 1 Male survival (in days) following experimental treatments in *Tenebrio molitor* adults: a) fungus-challenged males; b) negative control males; c) nylon-challenged males; and d) positive control males

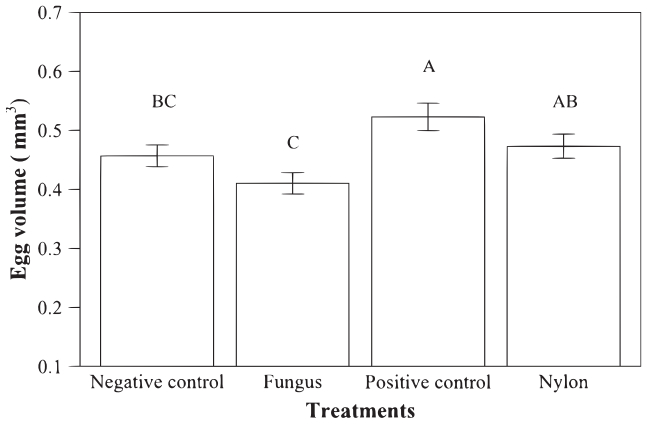

FIGURE 2 Egg volume (mean ±*SD*) from siring males after female preferences for four different male treatments in *Tenebrio molitor*. Means with different letters indicate significant differences

compared to eggs sired by males of the negative control group. Eggs from fungus group did not differ in lipid content from those of positive control ($z = 1.90$, $p = 0.22$) and implant ($z = 0.47$, $p = 0.96$).

In terms of egg number, there were significant differences among all treatments ($X^2 = 60.035$, $p < 0.001$; Figure 4). Negative control males sired more eggs than fungus males ($z = 35.95$, $p < 0.001$), positive control males ($z = 44.21$, $p < 0.001$), and nylon males ($z = 52.40$, $p < 0.001$).

Male treatment had an effect on eclosion success ($X^2 = 8.737$, $p < 0.05$). Eclosion was higher for eggs sired by negative control males compared to fungus ($z = 14.39$, $p < 0.001$), nylon ($z = 9.28$, $p < 0.001$), and positive control males ($z = -22.12$, $p < 0.001$). Eggs

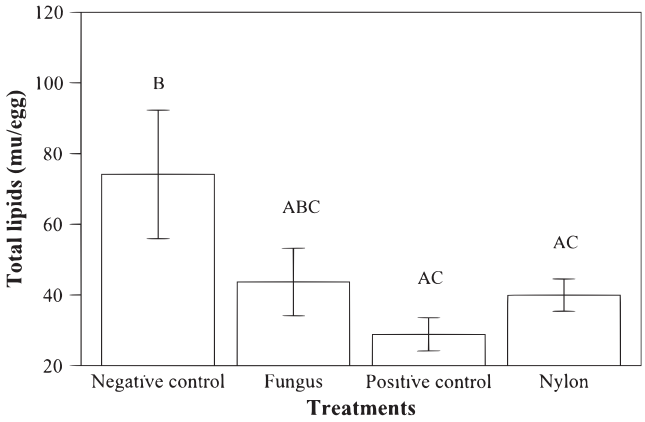

FIGURE 3 Total concentration of lipids (mean ±*SD*) from siring males after female preferences for four different male treatments in *Tenebrio molitor*. Means with different letters indicate significant differences

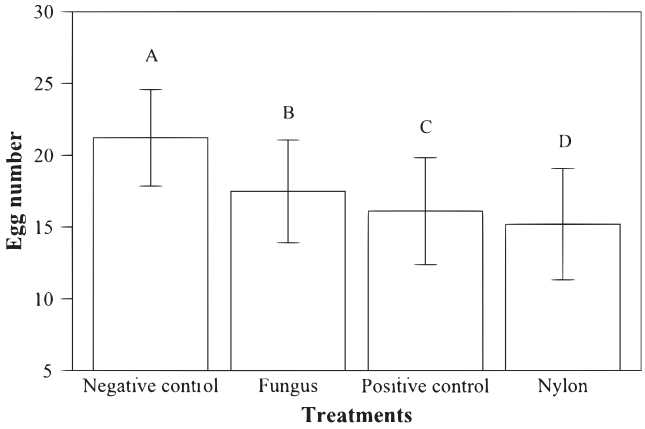

FIGURE 4 Number of eggs (mean ±*SD*) laid from siring males after female preferences for four different male treatments in *Tenebrio molitor*. Means with different letters indicate significant differences

from fungus males had a higher eclosion success than those from positive control males ($z = -9.28$, $p < 0.001$).

4 | DISCUSSION

Similar to some previous studies (Kivleniece et al., 2010; Krams et al., 2011; Nielsen & Holman, 2012; Sadd et al., 2006), *T. molitor* females showed a preference for sick males over healthy ones. Notice that this applies to situations when males were infected with fungus but not to males treated with nylons. In this extent, we used the same number of nylon implants that elicited an increase in pheromone production at the expense of immune ability by previous studies (Kivleniece et al., 2010; Krams et al., 2011). However, when comparing fungus-treated males versus nylon-challenged males, no difference emerged meaning that females were similarly attracted to both so that either male challenge elicited similar pheromone production. Contrary to our expectation, preferences expressed in all trials indicated that females chose challenged males no faster than

non-challenged males. This can be interpreted as females being able to detect and process scents at a similar rate for all treatments.

To complement our experimental manipulation of male condition, our protocol included the survival cost of challenges for all males. In this regard, we provided evidence for such negative effect: Fungus-treated males showed an impaired survival compared to nylon, positive, and negative control males. Interestingly, nylon implants did not lead to reduced survival compared to control groups. This lack of difference, as well as those anomalous results of female preferences expressed above, suggests that experiments using nylon implants must be interpreted with care (Moreno-García et al., 2013). One fundamental difference between a fungus pathogen and nylon implants is that the former can lead to a generalized weakening of the cuticle structure (e.g., Lacey, Lacey, & Roberts, 1988; Rangel, Alston, & Roberts, 2008; Wang & Leger, 2006) which is unlikely to be the case for the latter. These fungal effects may induce a resource allocation in the host to increase survival at the cost of activity and fecundity (Scholte, Knols, & Takken, 2006) and, in general, explain more costly effects compared to nylon challenges. Note that positive control males died at a similar rate than challenged males. A few studies using Tween 80 have also found negative effects on insect survival (e.g., Yazgan, 1981; Luz, Silva, Magalhães, Cordeiro, & Tigano, 1999; Enríquez-Vara, Córdoba-Aguilar, Guzmán-Franco, Alatorre-Rosas, & Contreras-Garduño, 2012). One explanation is that the Tween immersion that animals experienced may have obstructed some of the insect body openings such as the mouth or spiracles. This may have rendered Tween-treated animals to impair their feeding or respiration activities. This explanation means that Tween may be still a safe control but that the way this should be provided is not via immersion.

Why do females prefer mating with immune-challenged males? According to theory and in the face of an infection, the limited amount of resources that animals can secure would induce a trade-off between survival and other life-history traits, such as current reproductive effort (McKean & Lazzaro, 2011; Stearns, 1989). If the survival expectancy is perceived as low, animals should invest more in current reproduction as a last-ditch effort to maximize their fitness even if they die soon (Clutton-Brock, 1984; Kivleniece et al., 2010). This last strategy is known as terminal investment (Williams, 1966) and it can be elicited not only by pathogens, but also by any factor that reduces the residual reproductive value of an individual (e.g., Creighton, Heflin, & Belk, 2009; Heinze & Schrempf, 2012; Billman & Belk, 2014). So, possibly our challenged beetles may have re-allocated their resources to make them more attractive at the risk of surviving less in a terminal investment fashion. Evidence for the terminal investment comes not only from other animals (e.g., Copeland & Fedorka, 2012; González-Tokman, González-Santoyo, & Córdoba-Aguilar, 2013) but also our study species (Kivleniece et al., 2010; Krams et al., 2011). For example, experimentally infected male damselflies tend to defend their mating territories for longer compared to non-infected males (González-Tokman et al., 2013). Thus, infected males increased their chances to mate although they lived less than non-infected males (González-Tokman et al., 2013).

Assuming a terminal investment basis for our study species, there was a possible penalization for terminally investing males in terms of reduced fitness for offspring as fungus-treated males gave rise to smaller and fewer eggs. Furthermore, although there was difference in protein concentration across treatments, eggs sired by challenged males had less lipid content than those sired control males. Perhaps this lipid difference may explain why eggs from fungus-treated males had a reduced eclosion success. This lipid-based explanation may be related to spermatophore quality. Related to this, evidence from different insects indicate that spermatophores can serve as a lipid source for egg production, especially those lipids that cannot be synthesized such as cholesterol (reviewed by Marshall, 1982; Lewis & South, 2012). Thus, we suggest that terminally investing male beetles were penalized despite their large investment in attracting females. These ideas need to be formally tested.

Another explanation for why fungus-treated males were more attractive is that, rather than the insect, it is the fungus what makes the animal attractive. In fact, fungal manipulation of invertebrate hosts includes modification of the host's behavior (reviewed by Roy, Steinkraus, Eilenberg, Hajek, & Pell, 2006). For example, recent evidence has suggested that *Beauveria bassiana* entomopathogenic fungus is attractive to mosquitoes (George, Jenkins, Blanford, Thomas, & Baker, 2013). Also, fungus-killed female house flies are more attractive to males than non-infected females (Møller, 1993). Thus, fungal manipulation may induce fungus free animals to become attracted to fungus-infected animals (Roy et al., 2006). From the fungus perspective, this is an effective strategy to disperse and colonize new hosts (Watson & Petersen, 1993). One mechanism for this is that the fungus increases the insect's volatile production. If this is the case, then infected males may die sooner due to the a) cost of the infection and/or b) increased production of volatiles in a short period (although it may also be that it is the fungus the one that produces semiochemicals of sexual nature). Whatever the cost, it is clearly advantageous for the infected male as it becomes more effective at attracting females.

How are sick males attractive but then end up with reduced fitness? Sick males may indeed enhance their volatile production to attract females more intensively so that, according to theory, females take such production as an honest signal of male quality. Females may have used pheromone as a first trait to evaluate males to then correct their decision after copulation as implied by the differential allocation hypothesis. As a matter of fact, females of several species use a number of traits to have a "balanced" assessment of male quality (e.g., Hill, Enstrom, Ketterson, Nolan, & Ziegenfus, 1999; Hankison & Morris, 2003; Hasegawa, 2018) which is in agreement with theory (Candolin, 2003; Møller & Pomiankowski, 1993). Perhaps extended filters occur after copulation, whereby females assess other traits that may be coupled with male condition (e.g., Evans, Zane, Francescato, & Pilastro, 2003). One related example is that of *Tribolium castaneum* beetles whose females assess male condition during copulation (Fedina, 2007). However, cryptic female choice can be ruled out if males are able to modulate egg production by providing material benefits to females. As indicated before, this possibility can apply to *T. molitor* as males

provide a spermatophore whose quality may affect offspring traits (Drnevich, Papke, Rauser, & Rutowski, 2001; Worden & Parker, 2001). Thus, we hypothesize that variation in spermatophore quality may affect egg production and condition. How females gather such benefits from the spermatophore is unclear. One way is if females use the lipoprotein materials that conform the spermatophore wall (Gadzama & Happ, 1974), for egg production. According to this material benefits possibility, perhaps fungus-treated males re-allocated more resources to pheromone but ended up producing spermatophores of reduced quality that affected offspring' fitness. One way to look at this relation is testing whether pheromone production is directly related to spermatophore quality. There is evidence that pheromone production is costly (Harari, Zahavi, & Thiéry, 2011; Rantala, Kortet, Kotiaho, Vainikka, & Suhonen, 2003) but whether there is trade-off between this trait and spermatophore production is unknown.

In conclusion, our study indicates that *T. molitor* females were more attracted to sick males, but this led females to lay fewer and smaller eggs, and with less lipid content and eclosion success This negative fitness outcome may be due to either females corrected their choice by investing less in their eggs or that sick males provided fewer energetic resources to females. This second explanation is in agreement with the differential allocation hypothesis which predicts a positive correlation between male quality and maternal investment (Sheldon, 2000). The fact that sick males invested considerably more than healthy males, to attract females is also coherent with the terminal investment hypothesis (Williams, 1966).

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